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PLANTLET REGENERATION FROM SHOOT TIP CULTURE OF *PELARGONIUM ZONALE* HYBRID

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Introduction

Regeneration of plants in tissue culture provides a possibility for the production of large numbers of plantlets.

The possibility of regeneration of geranium plantlets by in vitro technique has been reported by Pillai and Hildebrandt (1969), Abo El-Nil and Hildebrandt (1973), Bennici (1974), Chen and Galston (1965, 1967), Skirvin and Janick (1976) and Debergh and Maene (1977). Virus and bacterial tested clones were obtained with *Pelargonium*, by using a culture of shoot and meristem tips (Pillai and Hildebrandt 1968a, c, 1969; Reuther 1975; Gippert and Schmelzer 1973; Hamdorf 1976, Chen and Galston 1965, 1967; Abo El-Nil and Hildebrandt 1973; Beauchesne et al. 1977; Feutry 1976; Theiler 1977; and Pastalka and Hildebrandt 1978).

Debergh and Maene's (1977) experiments performed with *P. hortorum* cvs. *'Block'* and *'Bundeskandsler'* have given a rapid clonal propagation method for geranium. However, it has been shown that the *Pelargonium* shoot tip culture of different varieties responds differently to the same constituents of nutrient media (Theiler 1977). It is, therefore, necessary to find the optimal inductive medium for each cultivar individually.

The present report describes our attempt at obtaining organogenic cultures (calliclones) from the shoot tip of two varieties of *Pelargonium zonale* hybrid by the Debergh and Maene's method.

Material and Methods

Seedlings, about 1 month old, of *Pelargonium zonale* hybrid cvs. *'Career'* and *'Fire flash'* (thus named and qualified by the grower — Žitnjak, Zagreb) were used for multiplication experiments. Surface sterilization of plant material was carried out for 20 minutes with 3% Halamid (99.5% p-toluen-sulfonchloramide as Na-salt), a commercial product of Pliva (Zagreb), and it was then washed 3 times with sterile water. Isolation of shoot tip explants was carried out under a stereomicroscope which showed that they consisted of the apical dome with one or two leaf primordia, and were about 0.5 mm high. Single explants were placed apex up on agar nutrient medium in test tubes (23 × × 120 mm).

The nutrient medium proposed by Debergh and Maene (1977) consisted of macroelements and FeEDTA, according to Murashige and Skoog (1962), microelements according to Nitsch and Nitsch (1970), and was supplemented with (mg l^{-1}): thiamine HCl 0.4; m-inositol 200; caseine hydrolysate 1000; sucrose 30,000; Difco bacto agar 6,000; indole-3-acetic acid (IAA) 0.5 and kinetin (K) 10.0. The pH of the medium was 5.8 before autoclaving. The medium was autoclaved at 15 lb/sq in for 15 minutes.

The cultures were permanently exposed to 1500 lx of artificial light (day-light fluorescent tubes), 16 hours light and 8 hours darkness daily at the temperature of 26°C.

The cultures were examined for *Xanthomonas pelargonii* by plating out homogenized sections of tissues onto the medium after Beauchesne et al. (1977). They were found to be free of X. *pelargonii* and other bacteria.

Results

Shoot tips, not longer than 0.5 mm (Fig. 1), consisting of the apex and 1—2 leaf primordia of *P. zonale* hybrid cvs. *'Career'* and *'Fire flash'* were cultured for 1 week on the basal medium without growth substances, and then transfereed on the medium with 0.5 mg l^{-1} IAA and 10 mg l^{-1}

Figs. 1—5.
Development of geranium plants *(P. zonale* hybrid cv. *'Fire flash')* from shoot tip callus culture.

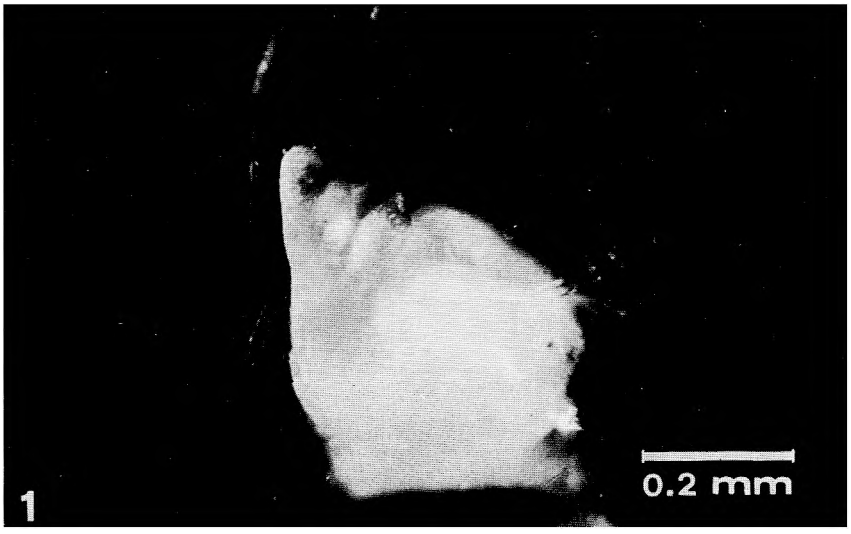
Fig. 1. Geranium shoot tip prepared for tissue culture.

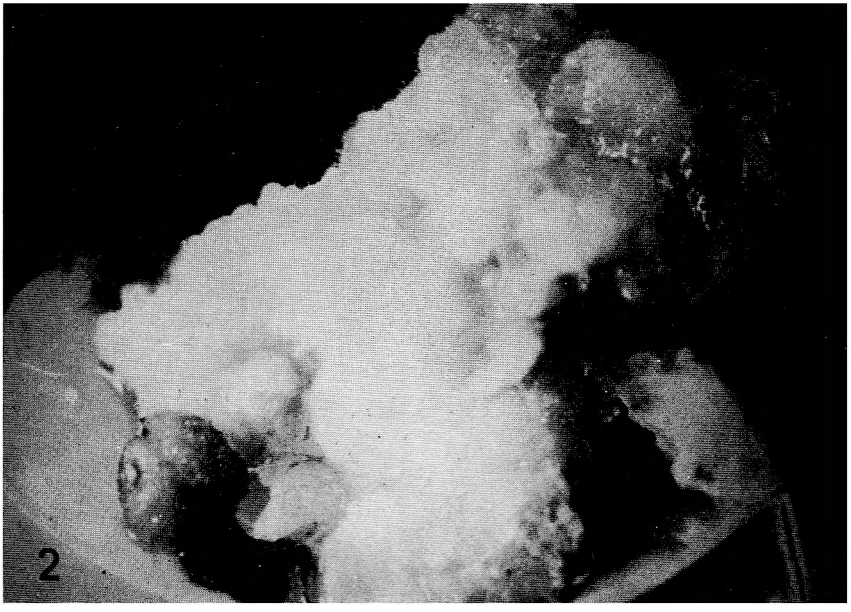
Fig. 2. Primary culture of stem tip with callus developed on modified MS medium with 0.5 mg l^{-1} IAA and 10 mg l^{-1} kinetin. x 4.4

Fig. 3. The 7th subculture of organogenic callus with regenerated shoots. x 4

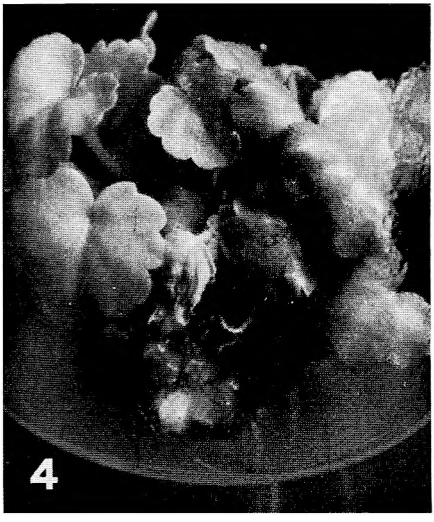
Fig. 4. Rooted plantlets after transfer on MS medium with 0.1 mg l^{-1} IAA. x 2

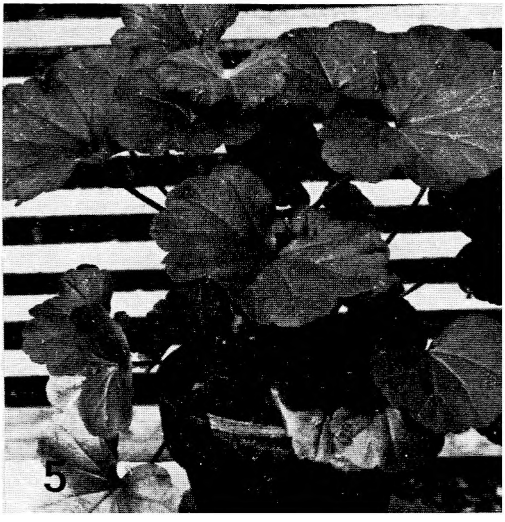
Fig. 5. In vitro regenerated grown-up geranium plant cv. *'Fire flash'*.





Figs. 1—2.





Figs. 3—5.

kinetin. None of the 24 inoculated shoot tips of the *'Career'* grew, but 30% of the *'Fire flash'* explants formed a well growing callus culture with caulogenic potentiality (Fig. 2).

By continuous subculturing every 4 weeks onto a fresh medium of the same composition, at an illumination regimen of 16 hours daily, the inocula containing shoots continued to grow and to form continuously adventive shoots during a period longer than a year (to the 9th subculture up to now).

It is significant, however, that one part of the cultures (about 30%) kept decaying after being subcultured, although all the inocula were uniformly prepared, i. e. of the same size and supplied with the same organic constituents. Further development of the newly formed shoots and their hardiness were easily maintained by transferring them onto a fresh basic medium with an addition of 0.1 mg l^{-1} IAA only (Fig. 4). After a month culturing at in illumination of 1200 lx 16 hours daily all plants took rootlets and were ready for potting. The plantlets were transferred to plastic pots in a sterile mixture of peat:perlit:soil (1:1:1); and were covered with a plastic bag to keep moisture for 2 weeks. After this stage, the plants grew vigorously, flowered and had the appearance of normal healthy plants.

Discussion

In the case of *Pelargonium* the differentiation and the growth possibility in a culture greatly depends on the cultivar (Pillai and Hildebrandt 1969, Skirvin and Janick 1976, Theiler 1977). The effect of plant growth regulators can also be different, depending on the cultivar (Beauchesne et al. 1977). Theiler's experiments (1977), as well, have clearly shown that geranium varieties respond very differently to their respective media, and that not all varieties can be grown easily.

In this work we used the medium proposed by Debergh and Maene (1977), in order to establish organogenic callus from stem tips of *Pelargonium zonale* hybrid 2 cvs. *'Career'* and *'Fire flash'*. The *'Career'* cultivar did not respond to in vitro culture conditions, while the 'Fire flash' did, but only in 30% of explants. This is considerably less than 80—90% of survived explants of *P. hortorum* cvs. *'Block'* and *'Bundeskandsler'* which were obtained by Debergh and Maene (1977). In our experiments we did not use a dark period for culture initiation; nevertheless, we obtained organogenic cultures, contrary to Pillai and Hildebrandt (1969). Out of 9 cultured varieties of *zonale* hybrids Pillai and Hildebrandt (1968 a, b, 1969) obtained only a low percentage of rooted shoots. The regenerated shoots in our experiments rooted very easily when transferred onto the basal medium with 0.1 mg l^{-1} IAA and exposed to 1200 lx of 16/8 h light dark photoperiods.

In addition to the regenerative capacity of a new cultivar we have tested, it would be advisable to test the variability of plants obtained by calliclones, because they could differ from the parental plants, as shown by the work of Skirvin (1978) and Skirvin and Janick (1976).

Summary

Shoot tips (0.5 mm high) of *Pelargonium zonale* hybrid cvs. *'Career'* and *'Fire flash'* were cultured on modified MS medium: macroelements (Murashige and Skoog 1962), microelements (Nitsch and Nitsch 1970) containing (mg l^{-1}) thiamine.HCl 0.4, m-inositol 200, caseine hydrolysate 1000, IAA 0.5, kinetin 10.0; (g l^{-1}) sucrose 30, bacto agar 6; pH 5.8.

The *'Career'* did not grow on this medium, while the *'Fire flash'* did: 30% of shoots produced an organogenic callus, which was capable of being continuously subcultured. The shoots and plantlets obtained rooted well on a medium containing 0.1 mg l^{-1} IAA only, and after a month they were transferred into pots. Callus cultures and in vitro formed plants, examined for *Xanthomonas pelargonii* and other bacteria, were found to be free of pathogens.

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SAŽETAK

REGENERACIJA BILJČICA U KULTURI VEGETACIJSKOG VRŠKA VRSTE *PELARGONIUM ZONALE* HYBRID

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Vegetacijske vrškove (veličine 0,5 mm) biljke *Pelargonium zonale* hybrid 2 sorte: *'Career'* i *'Fire flash'* kultivirali smo na modificiranom MS mediju (Makroelementi po Murashige i Skoogu 1962, mikroelementi po Nitsch i Nitschu 1970) uz dodane slijedeće tvari: (mg l^{-1}) tiamina.HCl 0,4; m-inositola 200; hidrolizata kazeina 1000; 0,5 kinetina 10,0 te (g l^{-1}) saharoze 30 i Difco bakto-agara 6; pH 5,8.

Dok sorta *'Career'* nije rasla na mediju ovog sastava, sorta *'Fire flash'* je na 30% eksplantata proizvela dobro rastući organogeni kalus koji se mogao supkultivirati. Izdanci i biljčice regenerirani in vitro dobro su se zakorjenjivali na osnovnom mediju uz dodatak samo 0,1 mg l^{-1} IAA i u velikom postotku preživljavali presađivanje u lonce.

Testiranje kalusnih kultura i in vitro regeneriranih biljaka na prisutnost bakterija *Xanthomonas pelargonii* i ostalih bakterija dalo je negativne rezultate.

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